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Patents ADP number (if you know it) 6055198002

If the applicant is a corporate body, give the country/state of its incorporation United Kingdom

4. Title of the invention BIOLOGICAL RESPONSE MODIFIERS

5. Name of your agent (if you have one)
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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BIOLOGICAL RESPONSE MODIFIERS

The present invention relates to biological response modifiers, which are formulations for use in treatment after exposure to radiation.

Biological response modifiers are given after radiation for the repair of tissue damage such as occurs during radiotherapy.

According to the present invention, we employ curcumin in a biological response modifier. Curcumin is diferuloylmethane and is present in the plant turmeric, *Curcuma longa Linn.* (Zingiberaceae). For the present invention, it is adequate to use an extract of turmeric.

The curcumin is used in an oil, preferably a natural plant oil, more preferably a vegetable oil and most preferably sunflower oil. Thus, in one aspect, the present invention provides a product comprising curcumin in such an oil. Typically the percentage by weight of curcumin in the oil is from 0.1%-60%, say from 0.5 to 25%, or about 1 to 20%.

We have found that the beneficial effects of curcumin in oil are enhanced by the inclusion of vitamin E, α-tocopherol. The ratio of curcumin:α-tocopherol is suitably in the range of 25:1 to 2.5:1, more preferably 20:1 to 5:1, most preferably about 10:1.

Thus, in one important aspect, the present invention provides a product which is a combination drug comprising curcumin and α-tocopherol in an oil, especially sunflower oil. Sunflower oil itself makes a significant contribution to the healing effects of the product.

The oil-based drug may be applied as a cream topically to an area of skin, mucus membranes or other tissues which have been exposed to radiation. Forms for topical administration include a mucus binding solution for oral mucosa, and a pessary for rectal administration. Alternatively, a formulation of the product suited for oral administration may be provided, notably capsules or tablet containing the product. Typically the amount of product taken daily by oral administration is 0.1g-20g per Kg body weight, say 0.25 to 10 g/Kg, or about 0.5 to 5 g/Kg.

The product of the present invention may be used for people who have been accidentally or unintentionally exposed to radiation, but it is a mainly expected to be of use for patients receiving radiation therapy. A specific application for the invention is therapy-induced mucositis, notabyl mucositis induced by radiotherapy and/or chemotherapy. A particular example involves mucositis caused by radiotherapy of the head and/or neck. Mucositis caused by conditions other than exposure to therapy can treated by the present invention.

The invention includes methods of treating a person exposed to radiation which includes administration of a curcumin and α-tocopherol. It is possible to separately administer the curcumin and α-tocopherol, but patient compliance is likely to be much better if a product of this invention is provided for administration.

Another method of this invention includes providing radiotherapy to a patient and administering curcumin and α-tocopherol. The radiotherapy can be as a single dose of radiation but will typically involve sequential exposure to doses of radiation, and the product of this

invention is administered either before or after first exposure to radiation and continued after the completion of radiotherapy. In a preferred method the product of this invention is administered after first exposure to radiation and continued after the completion of radiotherapy.

Typically the product of this invention is administered at repeated intervals following the end of exposure to radiation, and for example the product will be given for at least 14 or better 28 days or more following the last exposure to radiation.

Then invention also provides for the use of curcumin and α-tocopherol in the preparation of a medicament for the treatment of the effects of radiation, especially where the curcumin and α-tocopherol are in an oil such as sunflower oil.

The invention also includes treatment of mucositis and other conditions, including lesions in skin, the central nervous system (spinal chord and brain) or the gastrointestinal system. These other conditions may be induced by radiation. Examples of mucositis which can be treated include mucositis arising from exposure to radiotherapy, but the invention is not limited thereto, and conditions such as Irritable Bowel Syndrome can be treated. Thus, further aspects of this invention reside in methods of treating mucositis and other conditions with curcumin and α-tocopherol, compositions of curcumin and α-tocopherol for the treatment of mucositis and other conditions, and the use of curcumin and α-tocopherol in the preparation of medicaments for treating mucositis and other conditions. The methods of treatment extend to prophylactic treatments.

Further related aspects of this invention which can be achieved using curcumin and α-tocopherol include:

1- prevention of cancer, particularly development of radiotherapy-induced secondary tumours.

2- prevention and treatment of UV induced dermatitis (sunburn).

3- treatment of acute and chronic wounds on skin or buccal/oral mucosa and acceleration of healing of such wounds.

The invention embraces such methods, compositions for such methods, and the use of curcumin and/or α-tocopherol in the preparation of compositions for use in the methods.

More details of the present invention will be apparent from the following report of experimental work on Amelioration of Radiation-Induced Normal Tissue Reactions by a Combination Drug.

Claims then follow the experimental report.

Friday, March 22, 2002

Confidential

Amelioration of radiation-induced normal tissue reactions by a combination drug.

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Inroduction:

Approximately 50% of all cancer patients receive radiotherapy at one stage in their course of treatment. Local tumour control is directly related to the radiation dose. The greater the radiation dose the greater is the probability of tumour control. Although in theory it must be possible to eradicate a localised tumour with a large dose of radiation in practice the damage to normal tissues, which is also dose-dependent, is a major limiting factor. Therefore, the dose delivered to the tumour is always compromised for the danger of damaging normal tissues adjacent to the tumour.

Advances in the administration of radiation such as dose fractionation, low dose-rate irradiation, application of radiosensitisers or radioprotectors and better localisation techniques have increased the therapeutic gain. However, in reality, even with limited doses of radiation there are always relatively sensitive patients who may develop normal tissue reactions. Therefore, normal tissue damage is almost an inevitable consequence of radiotherapy in majority of cases. For example in radiotherapy of laryngopharyngeal tumours 95% of the patients might experience a kind of early transient reaction and 61% suffer more persistent late reactions (Rezvani et al, 1991).

Radiosensitizers or radioprotectors although modify the effects of radiation but they are implied to substances, which ought to be present at the time of irradiation. While these substances can be used for cases of planned irradiation such as radiotherapy, their use will be limited in post irradiation cases. These substances can not be used in the treatment of oversensitive patients who develop lesions as side effect of radiotherapy or for patients overexposed in radiation accidents. During last two decades, interventional treatments after radiotherapy and application of various biological response modifiers (BRMs) have been suggested. BRMs are substances that are administered after irradiation. While these

compounds are of a great value in the treatment of oversensitive or overexposed patients they can also be used in cancer therapy in order to reduce the adverse effects of radiotherapy. Ameliorating the risk of normal tissue damage by the BRMs will allow radiotherapist to apply larger doses. Thus increase the probability of tumour cure without increasing the risk of normal tissue morbidity. At present, there are no effective BRMs for the treatment of radiation-induced normal tissue lesions including those of skin, mucus membranes or CNS.

In a search for finding a suitable BRM a number of herbal extracts were tested by Dr Rezvani, Research Institute, University of Oxford, UK during last few years. These substances, which demonstrated a limited success, included oral administration of the extracts of Chinese anti-inflammatory herbs Scutellaria barbata, Paeonia lactiflora, Salvia miltiorrhiza, E zhu, Glycyrrhiza uralensis, Astragalus membranaceus, Lonicera japonica, Paeonia sufruticosa, Trichosanthes kirilowii. These extracts had no beneficial effect in controlling the incidence of radiation-induced moist desquamation of skin in a rat model but accelerated its healing (Rezvani et al, unpublished data). In another study the efficacy of extracts of Fagopyrum esculentum, Symphytum officinalis and Calendula officinalis, given orally, had no beneficial effect in controlling the incidence of radiation-induced moist desquamation. It appeared that these herbal extracts also accelerated the healing of the moist desquamation (Rezvani et al, unpublished data).

After testing numerous herbal extracts, a drug consisting of the extract of Curcuma longa significantly reduced the incidence of radiation-induced moist desquamation in the skin of rat foot.

Ammelioration of radiation induced skin lesions by extract of Curcuma longa:
Skin is an important tissue that is frequently exposed to radiation either accidentally or as a consequence of the treatment of cancer patients by radiotherapy. Acute radiation damage to the epidermis is related to the sterilisation of reproductive component of epidermis; stem cells. The full functional integrity of the skin will be preserved if there are sufficient surviving clonogenic cells within the basal layer, or within the shaft of hair follicles, to allow for rapid repopulation of the surface. The absence of cell production results in the development of epithelial denudation (moist desquamation). The development of radiation-induced moist desquamation of rat foot is an established model for the study of this lesion (Rezvani et al, 2002).

Material and methods:
Mature (26 weeks old) female Sprague-Dawley rats were used in this study. The animals were housed in groups of three per cage and received standard pellet diet food and water ad libitum. Both hind feet of each rat were irradiated, under anaesthesia, with a range of doses of ^{60}Co gamma rays, at a dose-rate of ~1.3 Gy/min. Initially, animals were anaesthetised in a perspex box, flushed with oxygen and 2-3% halothane. Pre-anaesthetised rats were then positioned in a perspex irradiation jig and anaesthesia was maintained by continuous flushing with oxygen and 1-1.5% halothane at a rate of 2 l/min. The foot to be irradiated was positioned into a slot in a circular perspex holder (1 cm thick, 11 cm diameter) located at the center of the jig. Rats were positioned radially

around this central perspex portion of the jig and nine animals were irradiated at each time. Irradiation schedules involved graded single doses of ^{60}Co γ rays to the left foot of the animal. Then the animals were randomised into two groups of "test" and "placeb". The animals in test group received 1 ml/day of an ethanolic extract of Curcuma longa (1:4, 25%) by oral gavage. Those in placebo group received similar volume of 25% ethanol in water by oral gavage. The feet were examined for the appearance of moist desquamation, daily, between 7 and 23 days after irradiation. Nine animals were used per each dose point. Quantal data for the incidence of moist desquamation were analysed using logit analysis and ED_{50} (±SE) values, the dose required to produce moist desquamation in 50% of irradiated feet, were obtained for both test and placebo groups. Dose modification factor (DMF) was obtained by dividing the ED_{50} value for test group to that of the placebo group. Data analysis was carried out by the SAS statistical package (SAS, 1989).

Results:

Fig. 1 shows the dose effect curves for the incidence of moist desquamation of rat foot skin after irradiation with graded doses of ^{60}Co γ rays for test and placebo groups. The ED_{50} value of 22.54 Gy, for the incidence of moist desquamation in the skin of rat foot in animals treated with Curcuma longa extract, was significantly ($p < 0.01$) higher than the value of 21.47 Gy, the ED_{50} for the incidence of moist desquamation in placebo group. This difference in ED_{50} values resulted in a DMF of 1.05.

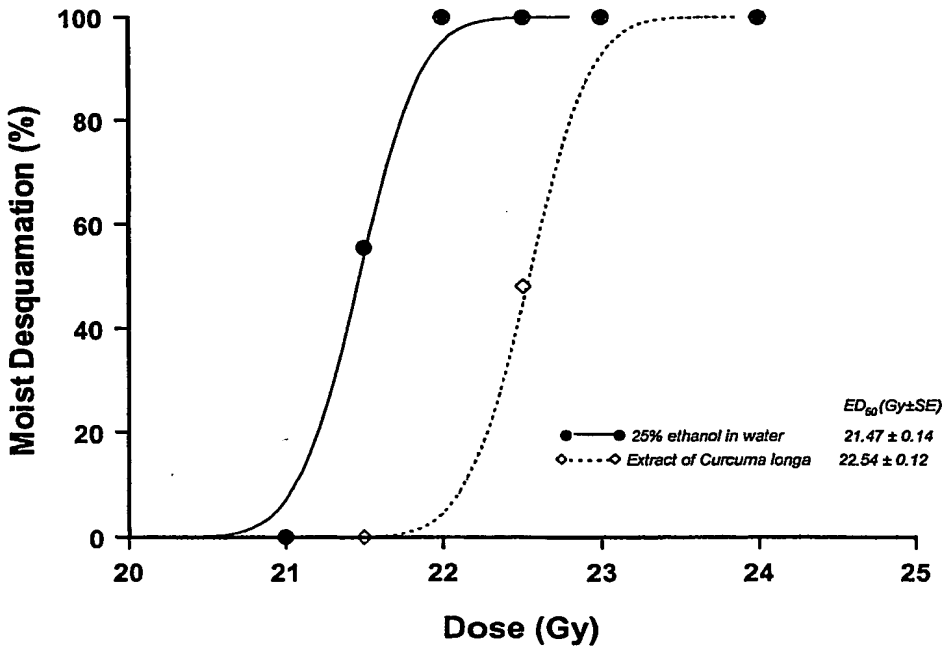

Fig. 1: Incidence of moist desquamation of rat foot skin after irradiation with graded doses of ^{60}Co γ rays.

Conclusion:
Although the modifying effect of extract of Curcuma longa was only 5% but it was very encouraging as this was the first time that the incidence of radiation-induced moist desquamation in this model could be influenced by an exogenous factor.

A compound consisting of ethanolic extracts of Curcuma longa and Euphorbia hirta was also used for the modification of radiation-induced skin lesions but with no success.

On the basis of this finding a combination of Curcumin (the active ingredient of the extract of Curcuma longa) and α-tocopherol dissolved in sun flower oil was tested. This compound demonstrated a significant beneficial effect in reducing the incidence of radiation-induced skin lesions and oral mucositis. Although sun flower oil was initially used as a solvent for Curcumin but during later experiments it was found that sun flower oil itself had an additional effect. Therefore, a combination drug (Compound A) consisting of Curcumin, α-tocopherol and sun flower oil (SFO) was developed and tested. The efficacy of this new compound in the treatment of radiation-induced normal tissue lesions is described here.

Components of Compound A:
Compound A consists of Curcumin, α-tocopherol and sunflower oil. The dosage used in experiments reported here is α-tocopherol 20 mg/kg/day, Curcumin 200 mg/kg/day in 0.5 ml/day SFO.

Sun flower oil:
There is no literature suggesting the involvement of sun flower oil in the treatment of radiation-induced lesions. However, in earlier studies at the Research Institute (Hopewell et al, 1994) it was reported that using sun flower oil as a placebo had some beneficial effects in modification of radiation-induced skin lesions in pigs.

α-tocopherol:
α-tocopherol (vitamin E) has not been used on its own in the treatment of radiation lesions but it was used as part of a combination treatment, adminstered for 8 weeks, 24 hours after irradiation of the skin of rabbits and showed no beneficial effect (Lefaix et al , 1992). However, α-tocopherol in combination with Pentoxyfilline was significantly effective in softening and shrinking of radiation-induced fibrotic scar in pig skin (Lefaix et al, 1999) and human (Delanian, 1998).

Curcumin:
Curcumin (diferuloyl methane) is a phenolic antioxidant and antiinflammatory available in the rhizome of the plant *Curcuma longa Linn.* (Zingiberaceae). This yellow phytochemical can be extracted from this plant with ethanol or other organic solvents. This chemical has strong antioxidant and free radical-scavenging activity (Rao et al, 1982; Kunchandy & Rao, 1990) and it inhibits lipid peroxidation (Joe & Lokesh, 1994; Sreejayan & Rao, 1994; Sreejayan et al, 1997), including radiation-induced lipid peroxidation (Sreejayan et al, 1997). Its anti-inflammatory action can be due to its inhibitory effect on arachidonic acid metabolism via lipoxygenase and cyclooxygenase pathways (Stoner & Mukhtar, 1995). It is involved with Hemox-1, it protects endothelial cells (Motterlini, et al, 2000) and it is a potent inhibitor of

mutagenesis and has demonstrated strong anticancer activity (Huang et al, 1988; Mehta & Moon, 1991; Nagabhushan & Bhide, 1992; Rao et al, 1993; Huang et al, 1994; Lu et al, 1994; Subramanian; 1994; Ramachandran & You, 1999; Inano & Onoda, 2002). Furthermore, reports indicate that curcumin inhibits the expression of c-fos, c-jun and c-myc proto-oncogenes (Rao et al, 1993; Huang et al, 1994; Lu et al, 1994; Subramanian; 1994; Chen & Tan, 1998). Biological and pharmacological properties of curcumin have been reviewed (Govindarajan, 1980; Tonnesen, 1988; Ammon & Wahi, 1990; 1991; Huang et al, 1992).

Curcumin, besides antioxidant activities, has shown to inhibit radiation induced protein kinase C activity (Varadkar et al, 2001). PKC inhibits the ceramide pathway which in turn inhibits apoptosis. Curcumin can potentially interfere with this pathway (Varadkar et al, 2001) and may cause tissue sensitisation.

Majority of the reports on Curcumin involve its anticancer activities (Inano and Onoda, 2002; Ramachandran and You, 1999; Araujo et al, 1999). There is no reports of the application of Curcumin for the treatment of radiation induced skin or oral mucosal lesions, however, administration of curcumin for two weeks prior to irradiation has successfully modified the radiation response assessed by the measurement of the glyoxalase activity in the liver and spleen of irradiated mice (Choudhary et al, 1999) and it has been shown to be effective in the repair of both oxidative and reductive damages to proteins and oxidized amino acids caused by radiation (Kapoor & Priyadarsini, 2001).

Curcumin is abundantly available in oriental diet, it is on the FDA's GRAS (generally recognised as safe) list and no LD_{50} has been reported for it. Doses as high as 500-2000 g/kg body weight have shown no toxicity when fed to animals (cats, dogs, pigs and monkeys) for 60 weeks (Bille et al, 1985; Varadkar et al, 2001).

Amelioration of radiation-induced mucositis:

Another major side effect of radiotherapy, particularly of head and neck cancers, is radiation-induced mucositis. With encouraging results of skin studies it was thought that Compound A could give beneficial results in the treatment of radiation-induced mucositis. This was based on the similarities in the etiology of these two lesions.

The stem cells of the epithelial lining of the oral mucosa are non-specifically affected by many anti-cancer agents including radiation. Mucositis induced by chemotherapy or radiotherapy is an important dose-limiting side effect of cancer therapy (Sonis, 1998). Radiation-induced mucositis of upper aerodigestive tract, in particular, is a major dose-limiting factor in the treatment of head and neck tumours. Apart from being a painful and distressing experience, radiation-induced mucositis coupled with the associated xerostomia may lead to poor oral hygiene and weight loss in head and neck cancer patients. A planned course of treatment may be interrupted to allow for the healing of this acute reaction. This may impair the outcome of treatment. In one study, mucosal reactions were seen in almost all patients (95%) treated for head and neck cancer (Rezvani et al, 1991) with 68% of the patients having 'ulceration or fibrinous reaction' of the mucosal membrane. In another study 'severe acute mucosal effects' were reported in 52% of the patients treated with radiotherapy for carcinoma of the oral cavity and the oropharynx (Maciejewski et al, 1990). Mucositis occurs in approximately 40% of cancer

patients treated with chemotherapy (Sonis, 1997) with 50% of them requiring modification of their cancer treatment and/or analgesia (Sonis, 1998).

A number of mouth washes containing antiseptic and/or analgesics have been used in the treatment of radiation-induced mucositis. These treatment approaches are ineffective in preventing the development of mucositis and have also been shown to be detrimental in some cases (Foote et al, 1994). Recently, a variety of novel therapeutic agents have been developed with limited success (Spandinger et al, 1994; Troussard et al, 1995; Farrell et al, 2000) with exception of keratinocyte growth factor where promising results have been reported (Doem et al, 2001). At present there is no effective method of treating radiation-induced mucositis.

Materials and methods:

On the basis of skin studies the extract of Curcuma longa was identified as a potential substance to modify radiation-induced oral mcositis. However, the active ingredient of this plant substance is curcumin (diferuloylmethane) which is commercially obtainable (Sigma-Aldrich). Therefore experiments were designed to test the effectiveness of curcumin in prevention of radiation-induced oral mucositis. It was also decided that adding α-tocopherol (5, 7, 8-Trimethyltocol) to curcumin might increase the beneficial effects of curcumin. However, the latter was not water soluble and it was decided that both curcumin and α-tocopherol could be mixed in sun flower oil. Therefore, in some studies (results not shown) sun flower oil (SFO) was used as placebo. However, when sun flower oil treated animals were compared with those that received water (as placebo) or no drug treatment at all it was found that sun flower oil itself had a beneficial effect in the treatment of radiation-induced oral mucositis and its addition enhanced the beneficial effects of curcumin and α-tocopherol . Therefore, a final compound was identified as combination of curcumin, α-tocopherol and sun flower oil. This compound (compound A) was used as a combination drug for the treatment of radiation -induced oral mucositis.

Mature (12 weeks old; 200-225g) female Sprague-Dawley rats were used for these studies. The animals were housed in groups of three per cage in conventional housing conditions, 55% humidity, 70-72 °F, 12 to12 hrs light-dark-cycle and received a standard pellet diet and water ad libitum. The animals were maintained, and all experimental procedures performed in compliance with the Animal (Scientific Procedures) Act 1986. While under anesthesia the animals tongue was slightly extended outside and a region of the underside of the tongue was irradiated in-situ with single doses of 2.27 MeV β-rays from a 5mm or 11mm diameter $^{90}Sr/^{90}Y$ plaque. The dose-rate of the 5mm $^{90}Sr/^{90}Y$ plaque was ~10Gy/min and that of 11mm source was ~3Gy/min at the surface of the mucus membrane. 5mm source was used for single dose studie and 11mm source was used for fractionated studies. The irradiation was carried out by simply positioning the sealed $^{90}Sr/^{90}Y$ plaque in contact with the surface of the tongue. The tongue was stretched gently and radioactive source was placed with a uniform pressure in all cases in order to avoid any local hypoxia. However, if in any unlikely situation local ischemia/hypoxia have occurred due to the stretching the tongue or pressure of the source the effect was ignored as this would have equally applied for both control and test animals. The irradiation site was medial to the sublingual veins and a 4 mm margin was maintained

from the tip of the tongue. Irradiations were carried out under general anesthesia maintained with Halothane/oxygen mixture.

Single dose studies:
A total of 144 rats were used for this part of study. Four groups of 36 animals were irradiated with single doses of either 13.5, 15, 16.5 or 18 Gy. Following irradiation the animals in each dose group were subdivided into four treatment subgroups of 9 rats to receive 0.5 ml per day of either Compound A, SFO, α-tocopherol or water by oral gavage until the end of experiments. Nine animals were used at each dose point in each treatment group. Mucosal ulceration (erosion of mucosal epithelium) was considered as an end-point and this is referred by radiation-induced mucositis in the context of present experiments. From the day after irradiation until any acute radiation-induced oral mucosal lesion had healed the animals tongue were assessed daily for the presence of radiation-induced mucositis (mucosal ulceration) under light anesthesia which was maintained by a 1.5% Halothane, oxygen mixture. Daily assessment of mucositis was carried out under ×2 magnifying glass with a cold light prior to oral gavaging. Quantal data for the incidence of radiation-induced mucositis were analysed using logit analysis and ED_{50} (±SE) values, the dose at which radiation mucositis (mucosal ulceration) was observed in 50% of irradiated tongues, were obtained. The dose modification factor (DMF) defined as the ratio of the dose of radiation to cause mucositis in 'active' agent group to that in 'placebo' group was calculated. Data analysis was carried out using SAS statistical package (SAS, 1989).

Results:
The incidence of radiation-induced oral mucositis (mucosal ulceration) in the tongue of rats are shown in Figure 2. There was a modest increase in the ED_{50} values after both α-tocopherol and SFO administration that resulted in DMF values of 1.05 and 1.04, respectively. The ED_{50} value of 18.16 ± 0.70 Gy obtained for the treatment of radiation-induced moist desquamation after treatment with compound A was significantly ($p<0.01$) higher than that of the animals that received water, SFO or α-tocopherol.

Discussion:
Both α-tocopherol and SFO showed a modest beneficial effect in the treatment of radiation induced oral mucositis. However, compound A significantly reduced the incidence of radiation mucositis with a signifcant DMF value of 1.24±0.06.

Mucosal ulceration (erosion of mucosal epithelium) was considered as an endpoint and it appeared to be a reliable representation of radiation-induced oral mucositis. Similar end point has been used by other authors (Doerr et al, 2001). The model involves the irradiation of only a small area of the underside of the tongue. This appears to be a good model to study the clinically relevant end point of oral mucositis following irradiation. While a good dose-response relationship was obtained for the incidence of mucositis the reaction had no apparent effect on the animals general wellbeing. There were no noticeable changes observed in animals with respect to body weight, eating, drinking or behavior.

While the intensity and duration of mucositis are dose dependent, the latent period for the development of mucositis will primarily depend on the turnover of the epithelial layer. In the present model the lesions developed from around 10 days after irradiation and the latency period was independent of the radiation dose and the treatment group. This supports the findings of Dörr et al (2001) who observed no dose dependency in the latency to the onset of oral mucositis in mice.

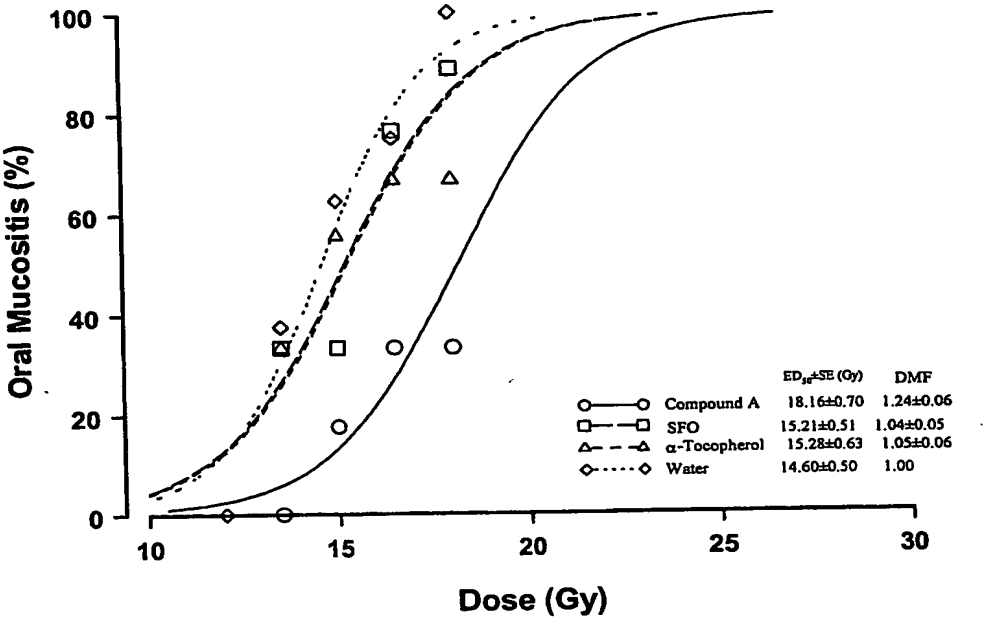

Fig. 2: Incidence of radiation-induced mucositis in rat after irradiation with graded doses of $^{90}Sr/^{90}Y$ beta rays and the effect of Compound A, SFO and α-tocopherol in amelioration of this lesion.

The basis for the classical management of therapy-induced mucositis is pain relief, prevention of dehydration, providing adequate nutrition and controlling infection, such as candidiasis (Symonds et al, 1996). A number of mouth washes containing antiseptic and/or analgesic agents have been developed and used but with no beneficial effects. This includes chlorhexidine and nystatin (Epstein et al, 1992), glycerine, thymol, glycerine, lemon and hydrogen peroxide (Feber, 1996). These classical treatment approaches not only are inefficient in preventing the development of mucositis but have been shown to be detrimental in some cases. This includes the application of sodium bicarbonate (DeWalt et al, 1969) and chlorhexidine (Foote et al, 1994). It appears that frequent mechanical cleansing of the mouth by sophisticated mouthwashes is harmful and that a simple saline solution is indicated as the most effective mouth wash in therapy-induced

oral mucositis (Symonds et al, 1998). However, damage to the parotid glands (Stephens et al, 1986) and disturbances in intercellular signaling (Coppers and Kampinga, 2001) after irradiation can result in a thickening of the saliva, a reduction in its production and dehydration of the mucus membrane. This can encourage infection which in turn will delay the healing of the ulceration. Based on this hypothesis some antibiotic based applications have proven to be beneficial in controlling mucositis (Spijkervet et al, 1991; Symonds et al, 1996).

The oral mucosa is a continuously renewing tissue consisting of a stratified squamous epithelium. Epithelial stem cells in the basal germinal layer proliferate, with a high rate of renewal, to balance the loss of cells from the surface layer. This rapid turnover of mucosal tissue renders this tissue responsive to both radiotherapy and chemotherapy. After irradiation while the cell loss continues from the superficial layers of the mucosa the deeper basal cells, killed or damaged by irradiation failing to produce replacements for the lost cells. Therefore, the mucosa becomes thinner and once the number of epithelial cells reach a critical level a broken mucosa develops as a result of the denudation. The protective effect of painting the mucosal surface with silver nitrate for several days before radiotherapy (Maciejewski et al, 1990), Interleukin-1 (Zaghloul et al, 1994), granulocyte colony-stimulating factor (Spadinger et al, 1994; Nicolatou et al, 1998), granulocyte macrophage colony-stimulating factor (Troussard et al, 1995) and keratinocyte growth factor (Farrel et al, 1999; Doerr et al, 2001) is possibly due to an increase in the number of mucosal cells (hyperplasia) prior to or during radiotherapy treatment.

An alternative hypothesis in the treatment of treatment-induced mucositis is the use of substances that promote healing of the ulcerated mucosa and coating the mucus membrane to prevent further damage by the use of mucus binding substances. Sucralfate, which forms a barrier on the mucosa, has been shown to reduce the pain associated with mucositis (Allison et al, 1995). There are conflicting reports on the ability of sucralfate in prevention of mucositis (Epstein et al, 1992; Cengiz et al, 1999; Etiz et al, 2000).

Fractinated dose studies:
Fractionated radiotherapy is employed in curative treatment, therefore, biological response modifiers need to be assessed in relation to fractionated schedules. The results of single dose studies cannot be translated for clinical use, particularly for dose escalation purpose in radiotherapy, unless further results involving multiple small fractions, comparable to radiotherapy regimens used for the treatment of cancer patients, are made available. The usual fractionated radiotherapy consists of 25 fractions of 2 Gy delivered daily, 5 days per week. Such a schedule will be completed in 33 days. In a rat model, where radiation induced musositis develops from around 10 days after irradiation, 25 fractions will span over the period of the development of mucosistis. A schedule involving a short overall treatment time will be required in this model. The most appropriate and established technique, which closely mimics clinical practice, consists of a limited number of 2 Gy fractions followed by a large top-up dose.

Materials and method:

A total of 126 rats were used for this part of study. Three groups of 36 and one group of 18 animals were irradiated with eight daily fractions of 2 Gy (5 fractions per week) followed by single top-up doses various size (7.5-17.5 Gy). The first fraction was always started on a Monday and top-up dose was delivered on Thursday of the following week. Following irradiation the animals in each dose group were subdivided into four treatment subgroups. Nine animals were used at each dose point in each treatment group. Group 1 (Radiation only) received no further treatment except radiation. There were 36 (4x9) animals in this group. Group 2 (water) received 0.5 ml per day of water by oral gavage. There were only 18 (2x9) animals in this group. Group 3 (SFO) received 0.5 ml per day of sunflower oil. Group 4 (Compound A) received 0.5 ml per day of Compound A. Tested substances and placebo (water) were administered daily by oral gavage starting after the first 2 Gy fraction and continued until the end of experiments. Mucosal ulceration (erosion of mucosal epithelium) was considered as an end-point and this is referred by radiation-induced mucositis in the context of present experiments. From the day after start of irradiation until any acute radiation-induced oral mucosal lesion had healed the animals tongue were assessed daily for the presence of radiation-induced mucositis (mucosal ulceration) under light anesthesia which was maintained by a 1.5% Halothane, oxygen mixture.

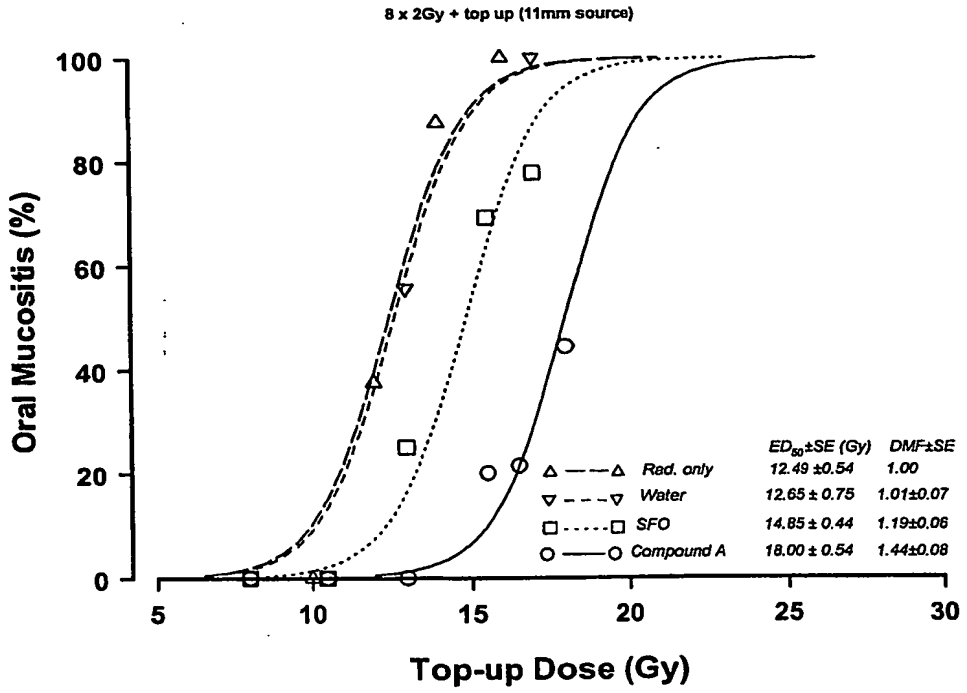

Fig. 3: Incidence of radiation-induced mucositis in rat after irradiation with with eight daily fractions of 2 Gy (5 fractions per week) followed by a single top-up dose. Total dose=8x2Gy+Top-up dose. Test substances, Compound A and SFO, and placebo (water) were given in 0.5 ml volumes starting after the first 2 Gy per fraction and continued until the end of experiments. Animals in Rad. Only group did not receive any treatment other than irradiation.

Daily assessment of mucositis was carried out under ×2 magnifying glass with a cold light prior to oral gavaging. Quantal data for the incidence of radiation-induced mucositis were analysed using logit analysis and top-up ED_{50} (±SE) values, the top-up dose at which radiation mucositis (mucosal ulceration) was observed in 50% of irradiated tongues, were obtained. The dose modification factor (DMF) defined as the ratio of the top-up dose of radiation to cause mucositis in test group to that in radiation only group was calculated. Data analysis was carried out using SAS statistical package (SAS, 1989).

Results:
The incidence of radiation-induced oral mucositis (mucosal ulceration) in the tongue of rats after fractionated irradiations are shown in Figure 3. There was no significant difference in the incidence of radiation-induced mucositis in water treated (placebo) animals and radiation only group. However, the ED_{50} values of 14.85± 0.44 Gy and 18.00± 0.08 Gy for the incidence of radiation-induced mucositis in SFO and Compund A groups, respectively were significantly higher than that of the radiation only and placebo groups. This resulted in significant DMF values of 1.19 ± 0.06 and 1.44 ± 0.08, respectively.

Conclusions:
Fractionated studies support the results of single dose study and further reveal the beneficial effect of both Compound A and SFO in the treatment of radiation-induced oral mucosistis. In fact the effect of both substances is enhanced after fractionated irradiations which is reflected by DMF values of 1.44 and 1.19, respectively.

Comparison of the efficacy of the components of Compound A:

Materials and method:
A total of 36 rats were used for this part of study. All animals were irradiated with eight daily fractions of 2 Gy (5 fractions per week) followed by single top-up of 16.5 Gy. The first fraction was always started on a Monday and top-up dose was delivered on Thursday of the following week. Following irradiation the animals were subdivided into four treatment groups of nine rats. Group 1 (Compound A) received 0.5 ml per day of Compound A. Group 2 (SFO+Curc.) received 200mg/kg/day Curcumin in 0.5 ml SFO. Group 3 (SFO+Toco.) received 20 mg/kg/day α-tocopherol in 0.5 ml SFO. Group 4 (Toco+Curc.) received 20 mg/kg/day α-tocopherol and 200 mg/kg/day Curcumin in 0.5 ml water. Tested substances were administered daily by oral gavage starting after the first 2 Gy fraction and continued until the end of experiments. Mucosal ulceration (erosion of mucosal epithelium) was considered as an end-point and this is referred by radiation-induced mucositis in the context of present experiments. From the day after start of irradiation until any acute radiation-induced oral mucosal lesion had healed the animals tongue were assessed daily for the presence of radiation-induced mucositis (mucosal ulceration) under light anesthesia which was maintained by a 1.5% Halothane, oxygen mixture. Daily assessment of mucositis was carried out under ×2 magnifying glass with a cold light prior to oral gavaging.

Results:
Table I shows the effect of Compound A and its components on the incidence of radiation-induced oral mucositis in rats irradiated with 8x2Gy daily fractions followed by a top-up dose of 16.5 Gy. Different combinations of the components of Compound A show a degree of effect in reducing the incidence of radiation-induced oral mucositis but the biggest effect is produced by the Compound A itself which contains all components.

Table I: Influence of Compound A and its components on the incidence of radiation-induced oral mucositis in rats irradiated with 8x2Gy daily fractions followed by a top-up dose of 16.5 Gy.

Modifying agent	Incidence of oral mucositis (%) (responder/at risk)
Compound A	11.11% (1/9)
SFO+Curcumin	66.67% (6/9)
SFO+α-tocopherol	33.33% (3/9)
α-tocopherol +Curcumin	55.56% (5/9)

Fischer's exact test revealed that the incidence rates shown in Table I were significantly different ($p<0.05$). This implies that all three components of Compound A are making contribution in alleviating the incidence of radiation-induced oral mucositis in rat.

General Conclusions and future work:
Compound A has proved beneficial in the treatment of radiation induced skin and oral mucosal damage. It appears to have an enhanced effect after fractionated studies, which is more relevant to clinical applications of radiotherapy in human patients. This compound has remarkable potentials in the treatment of radiation induced lesions of skin and oral mucosa. This is reflected in its remarkable DMF value of 1.44. Furhtermore, all components of this compound are non-toxic and the main ingredient of this compound Curcumin has demonstrated anticancer activities which makes the compound an ideal drug for the treatment of radiation-induced skin and mucosal lesions in cancer patients.

Further works are being carried out to assess the efficacy of Compound A in the treatment of radiation-induced lesions in central nervous system (radiation myelopathy) and gastrointstinal system.

References:

Allison RR, Vongtama V, Vaughan J, Shin KH. Symptomatic acute mucositis can be minimised or prophylaxed by the combination of sucalfate and fluconazole. Cancer Invest 1995; 13: 16-22.

Ammon H.P.T., Wahl M.A. (1990) Pharmacology of *Curcuma longa*. Planta. Med. 57: 1-7.

Araujo M.C.P., da Luz Dias F., Takahashi C.S. (1999) Potentiation by Turmeric and Curcumin of γ-Radiation-Induced Chromosome Aberrations in Chinese Hamster Ovary Cells Teratogen., Carcinogen., and Mutagen. 19:9-18.

Bille N., Larsen J.C., Hansen E.V., Wurtzen G. (1985) Subchronic oral toxicity of turmeric oleoresin in pigs. Food Chem. Toxicol. 23:967-73.

Cengiz M, Ozyar E, Ozturk D, Akyol F, Atahan IL, Hayran M. Sucralfate in the prevention of radiation-induced oral mucositis. J Clin Gastroenterol 1999;28:40-43.

Chen Y-R., Tan T-H. (1998) Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. Oncogene 17: 173-178.

Choudhary D., Chandra D., Kale R.K. (1999) Modulation of radioresponse of glyoxalase system by curcumin. J. Ethnopharmacol. 64: 1-7.

Coppes RP, Kampinga HH. Early radiation-induced loss of rat salivary gland function is not due to apoptotic or necrotic cell death but to impaired intracellular signalling. EURADOS/EULEP Newsletter, 2001; 9:2-3.

Delanian S (1998) Striking regression of radiation-induced fibrosis by a combination of pentoxifylline and tocopherol. British Journal of Radiology 71: 892-894

Delanian S, Balla-Mekias S and Lefaix J-L (1999) Striking regression of chronic radiotherapy damage in a clinical trial of combined pentoxifylline and tocopherol. J. Clin. Oncol. 17: 3283-3290

DeWalt EM, Haines AK. The effects of specified stressors on healthy oral mucosa. Nurs Res 1969;18:22-27.

Dörr W, Noack R., Spekl K, Farrell CL. Modification of oral mucositis by keratinocyte growth factor: single radiation exposure. Int J Radiat Biol 2001; 77: 341-347.

Epstein JB, Vickars L, Spinelli J, Reece D. Efficacy of chlorhexidine and nystatin rinses in prevention of oral complications in leukemia and bone marrow transplantation. Oral Surg Oral Med Oral Pathol 1992;73:682-9.

Etiz D, Erkal HS, Serin M, et al. Clinical and histopathological evaluation of sucralfate in prevention of oral mucositis induced by radiation therapy in patients with head and neck malignancies. Oral Oncol 2000;36:116-20.

Farrell CL, Rex KL, Kaufman SA, et al. Effects of keratinocyte growth factor in the squamous epithelium of the upper aerodigestive tract of normal and irradiated mice. Int J Radiat Biol 1999;75: 609-620.

Feber T. Management of mucositis in oral irradiation. Clin Oncol (R Coll Radiol) 1996;8:106-11.

Foote RL, Loprinzi CL, Frank AR, et al. Randomized trial of a chlorhexidine mouthwash for alleviation of radiation-induced mucositis. J Clin Oncol 1994;12:2630-3

Govindarajan V.S. (1980) Turmeric chemistry, technology and quality. CRC Rev. Food Sci. Nutr. 12: 199-301.

Hopewell J.W., van den Aardweg G.J.M.J, Morris G.M., Rezvani M., Robbins M.E.C., Ross G.A. and Whitehouse E.M. (1994) Unsaturated lipids as modulators of radiation damage in normal tissues. *In: New approaches to cancer therapy: unsaturated lipids and photodynamic therapy. Ed. D.F. Horrobin, Churchill Communications, Europe (London) pp 88-106.*

Huang M-T., Smart R.C., Wong C-Q., Conney A.H. (1988) Inhibitory effect of curcumin, chlorogenic acid, caffeic acid and ferulic acid on tumor promotion in mouse skin by 12-O-tetradecanoylphorbol-13-acetate. Cancer Res. 48: 5941-5946.

Huang, M-T., Robertson F.M., Lysz T., Ferraro T., Wang Z.Y., Georgiadis C.A., Laskin J.D., Conney A.H. (1992) Inhibitory effects of curcumin on carcinogenesis in mouse epidermis. In: Huang M-T., Ho. C-T., Lee C.Y. (eds): "Phenolic Compounds in Food and Their Effects on Health II: Antioxidants and Cancer Prevention". Washington, DC: American Chemical Society, pp 339-349.

Huang M-T., Lou Y-R., Ma W., Newmark H.L., Reuhl K.R., conney A.H. (1994) Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer Res 54: 5841-5847.

Inano H. and Onoda M. (2002) Prevention of radiation-induced mammary tumors. Int. J. Radiat. Oncology Biol. Phys. 52: 212-223.

Joe B., Lokesh B.R. (1994) Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. Biochem. Biophys. Acta 1224: 255-263.

Kapoor S., Priyadarsini K.I. (2001) Protection of radiation-induced protein damage by curcumin. Biophysical Chem. 92: 119-126.

Kunchandy E., Rao M.N.A. (1990) Oxygen radical scavenging activity of curcumin. Int. J. Pharmaceut. 58: 237-240.

Lefaix JL, Daburon F and Tricaud (1992) Evolution radiopathologique spontanee et apres traitement medical dans deux modeles d'accident d'irradiation localisee. Bull Cancer/Radiother 79:189-198

Lefaix J-L, Delanian S, Vozenin M-C, Leplat J-J, Tricaud Y and Martin M (1999) Striking regression of subcutaneous fibrosis induced by high doses of gamma rays using a combination of pentoxifylline and alpha-tocopherol: an experimental study. Int. J. Radiat. Oncol. Biol. Phys. 43: 839-847

Lu Y.P., Chang R.L., Lou Y.R., Huang M-T., Newmark H.L., Reuhl, K.R., Conney A.H. (1994) Effect of curcumin on 12-tetradecanoylphorbol-13-acetate- and untraviolet B light-induced expression of c-Jun and c-Fos in JB6 cells and in mouse epidermis. Carcinogenesis 15: 2363-2370.

Maciejewski B, Withers HR, Taylor, JMG, Hliniak, A. Dose fractionation and regeneration in radiotherapy for cancer of the oral cavity and oropharynx. Part 2. Normal tissue response: acute and late effects. Int J Radiat Oncol Biol Phys 1990; 18: 101-111.

Mehta R.G., Moon R.C. (1991) Characterization of effective chemopreventive agents in mammary gland *in vitro* using an initiation-promotion protocol. Anticancer Res. 11: 593-596.

Motterlini R., Foresti R., Bassi R. and Green CJ (2000) Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radic. Biol. Med. 25:1303-1312.

Nagabhushan M., Bhide S.V. (1992) Curcumin as an inhibitor of cancer. J.Am. coll. Nutr. 11:192-198.

Nicolatou O, Sotiropoulou Lontou A, Skarlatos J, Kyprianou K, Kolitsi G, Dardoufas K. A pilot study of the effect of granulocyte-macrophage colony-stimulating factor on oral mucositis in head and neck cancer patients during X-radiation therapy: a preliminary report. Int J Radiat Oncol Biol Phys 1998;42:551-556.

Ramachandran C. and You W. (1999) Differential sesitivity of human mammary epithelial and breast carcinoma cell lines to curcumin. Breast Cancer Research and Treatment 54: 269-278.

Rao T.S., Basu N., Siddiqui H.H. (1982) Anti-inflammatory activity of curcumin analogues. Indian J. med. Res. 75: 574-578.

Rao C.V., Simi B., Reddy B.S. (1993) Inhibitory effect by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. Carcinogenesis 14: 2219-2225.

Rezvani M, Alcock CJ, Fowler JF, Haybittle JL, Hopewell JW, Wiernik G, Normal tissue reactions in the British Institute of Radiology study of 3 fractions/week versus 5

fractions/week in the treatment of carcinoma of the laryngo-pharynx by radiotherapy. Brit J Radiol 1991;64:1122-1133.

Rezvani M., Ross G., Morris G. M., Wilkinson J. H. and Bywaters A (2002) Evidence for humoural effects on the radiation response of rat foot skin. BJR 75: 50-55.

SAS/STAT User's Guide, ver. 6, 4th edn. Cany: SAS Institute, 1989.

Sonis ST. Oral mucositis. In: JF Holland et al. Cancer Medicine, 4th Edition, Philadelphia: Williams & Wilkins, 1997. pp. 3255-3264.

Sonis ST. Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity. Oral Oncol 1998;34:39-43.

Spadinger BG, Hodges E, Ruby E, Stanley R, Coccia P, Effect of granulocyte-macrophae colony stimulating factor on oral mucositis after haematopoietic stem cell transplantation. J. Clin. Oncol. 1994;12: 1917-1922.

Spijkervet FK, Van Saene HK, Van Saene JJ, et al. Effect of selective elimination of the oral flora on mucositis in irradiated head and neck cancer patients. J Surg Oncol 1991;46:167-73.

Sreejayan N., Rao M.N.A. (1994) Curcuminoids as potent inhibitors of lipid peroxidation. J. Pharm Pharmacol. 46: 1013-1016.

Sreejayan N., Rao M.N.A., Priyadarsini K.I., Devasagayan T.P.A. (1997) Inhibition of radiation-induced lipid peroxidation by curcumin. Int. J. Pharm. 151: 127-130.

Stephens LC, Ang KK, SchultheissTE, KingGK, Brock WA, Peters LJ. Target cell and mode of radiation injury in rhesus salivary glands. Radiother & Oncol. 1986; 7: 165-174.

Stoner G.D., Mukhtar H. (1995) Polyphenols as Cancer Chemopreventive Agents. J. Cell. Biochem. Suppl. 22: 169-180.

Subramanian M., Sreejayan, N., Rao M.N.A., Devasagayam T.P., Singh, B.B. (1994) Diminution of singlet oxygen-induced DNA damage by curcumin and related antioxidants. Mutation Res. 311: 249-255.

Symonds RP, McIlroy P, Khorrami J, et al. The reduction of radiation mucositis by selective decontamination antibiotic pastilles: a placebo-controlled double-blind trial. Br J Cancer 1996;74:312-7.

Symonds RP. Treatment-induced mucositis: an old problem with new remedies. Br J Cancer 1998;77: 1689-1695.

Tonnesen H.H. (1988) Chemistry, stability and analysis of curcumin, a naturally occurring drug molecule. Ph.D. Dissertation, The Institute of Pharmacy, University of Oslo.

Troussard X, Macro M, Vie B, et al. Human recombinant granulocyte-macrophage colony stimulating factor (hrGM-CSF) improves double hemibody irradiation (DHBI) tolerance in patients with stage III multiple myeloma: a pilot study. Br J Haematol 1995;89:191-5.

Varadkar P., Dubey P., Krishna M., Verma N.C. (2001) Modulation of radiation-induced protein kinase C activity by phenolics. J. Radiol. Prot. 21: 361-370.

Zaghloul MS, Dorie MJ, Kallman RF. Interleukin 1 increases thymidine labeling index of normal tissues of mice but not the tumor. Int J Radiat Oncol Biol Phys 1994;29:805-11.

Claims

1. The combination of curcumin and α-tocopherol.

2. The combination of claim 1, in the treatment of the effects of exposure to radiation.

3. The combination of claim 1, in the treatment of mucositis.

4. A formulation of curcumin and α-tocopherol.

5. A formulation according to claim 4, where the curcumin and α-tocopherol are in an oil.

6. A formulation according to claim 5, wherein the oil is sunflower oil.

7. A formulation according to any of claims 4 to 6, in a dosage form suited for topical or oral administration.

8. A method of treating a person exposed to radiation which involves administering a formulation according to any of claims 4 to 7.

9. A method according to claim 8, wherein the patient is receiving a course of radiotherapy.

10. The use of curcumin or α-tocopherol in the preparation of a medicament for a method of treating a person exposed to radiation by administering curcumin and α-tocopherol.

11. A method of treating mucositis which involves administering a formulation according to any of claims 4 to 7.

12. The use of curcumin or α-tocopherol in the preparation of a medicament for a method of treating mucositis by administering curcumin and α-tocopherol.

13. A method using using curcumin and α-tocopherol for:

1- prevention of cancer, particularly development of radiotherapy-induced secondary tumours; or

2- prevention and treatment of UV induced dermatitis (sunburn); or

3- treatment of acute and chronic wounds on skin or buccal/oral mucosa and acceleration of healing of such wounds.